

# Antioxidant-rich microalgal supplementation influences early post-settlement growth and heat-stress responses in *Acropora* recruits cultured ex situ in Palau

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## Abstract

Improving the survival of sexually propagated coral recruits through thermal stress remains a key bottleneck for scaling reef-restoration approaches. We tested whether dietary microalgal supplementation during early grow-out alters subsequent bleaching and mortality trajectories during a controlled cumulative heatwave emulation, and whether responses depend on species. *Acropora tenuis* and *Acropora digitifera* recruits were reared for ~3 months in flow-through tanks under four regimes: Spirulina (antioxidant-rich), Isochrysis, a mixed Isochrysis + Spirulina diet, and an unfed control relying on ambient plankton. Recruits were then exposed to a stepwise warming protocol and scored repeatedly for bleaching status and survival. In *A. tenuis*, feeding history significantly affected bleaching dynamics (likelihood ratio tests,  $p < 0.01$ ), with Spirulina-fed recruits showing delayed bleaching onset but more rapid progression once bleaching began, whereas Isochrysis-fed recruits were not detectably different from controls. In contrast, *A. digitifera* bleaching trajectories were not detectably influenced by feeding regime (overall treatment effects  $p > 0.05$ ), indicating a species-specific carry-over effect of diet on heat-stress response. During the grow-out phase, diet also affected early growth in *A. tenuis* (Type III test for treatment,  $p = 9.66 \times 10^{-7}$ ): model-based mean absolute growth was highest under Spirulina ( $0.306 \pm 0.022$  cm; 95% CI 0.263–0.349) and lower in controls ( $0.184 \pm 0.022$  cm) and mixed diets ( $0.139 \pm 0.022$  cm); treatment effects on *A. digitifera* growth were not detected ( $p = 0.368$ ). Mortality increased over the course of the heatwave emulation, but final mortality did not show a consistent reduction with feeding history. Together, these results show that practical nutritional interventions can improve early growth and modify the timing and pathway of bleaching in a species-dependent manner without necessarily improving ultimate

survival, emphasizing the need to evaluate both production-phase performance and heatwave response when optimizing recruit husbandry for restoration.

Keywords: coral recruits; early life stages; heterotrophy; nutrition; Spirulina; Isochrysis; oxidative stress; bleaching; restoration

## Introduction

Mass coral bleaching driven by marine heatwaves is now a dominant cause of reef degradation worldwide. Repeated bleaching events are compressing recovery windows and eroding reef-building capacity (Eakin et al., 2019). Bleaching can be divided into three processes: *(i)* Breakdown of Symbiosis: Bleaching is the physical manifestation of the partnership between the coral host and its Symbiodiniaceae (algae) collapsing. *(ii)* Reactive Oxygen Species (ROS) Accumulation and Oxidative Stress: Thermal and light stress damage the algal photosynthetic machinery (specifically Photosystem II), causing an overproduction of ROS. When ROS levels exceed the capacity of the host's antioxidant systems, oxidative stress occurs, damaging vital cellular components like DNA, lipids, and proteins. *(iii)* Failure of Symbiosis: The accumulation of ROS acts as a cellular signal that triggers the immune-like rejection of the algae. This results in the final breakdown of the coral–Symbiodiniaceae symbiosis, leading to the expulsion of the algae and the characteristic "bleached" appearance of the coral (Downs et al., 2002; Lesser, 2006; Rädecker et al., 2021).

Active restoration strategies increasingly aim to rebuild coral populations by enhancing recruitment. This is carried out either by seeding large numbers of larvae onto reefs or by culturing recruits ex situ and outplanting juveniles once they pass the most vulnerable early bottlenecks. However, post-settlement mortality and slow early growth can substantially reduce yield (proportion of corals that survive grow-out) increasing the labor and infrastructure required to produce viable outplants (Guest et al., 2014; dela Cruz et al., 2020).

One practical lever to improve recruit performance is to enhance nutrition: corals can capture suspended particles and dissolved organics, and these heterotrophic inputs can subsidize energy and essential nutrients when autotrophic supply is limited or when metabolic demands rise under stress (Houlbrèque and Ferrier-Pagès, 2009; Grottoli et al., 2006). It has been shown that in ex situ propagation supplemental feeding can improve survival and growth in juvenile stages (Conlan et al., 2017; Cooper et al., 2025). Dietary history can influence downstream stress trajectories by altering energetic reserves and physiological condition.

Most feeding studies in early life stages have focused on zooplankton or particulate diets. However, microalgae are attractive for scalable culture systems because they can be

produced in large volumes, delivered as standardized suspensions, and may supply micronutrients and bioactive compounds. Studies have shown that diet composition can influence coral physiology and performance, and different microalgal components can yield different outcomes (Conlan et al., 2019; Leal et al., 2014). Complementary work has also shown that larval feeding protocols can enhance settlement (Rodd et al., 2022) and that specific nutrients such as sterols can be critical for early life-stage performance and thermal tolerance (Matthews et al., 2025; Rodd et al., 2025).

Because oxidative stress is frequently associated with bleaching and with cellular damage under heat stress, antioxidant availability has been proposed as an additional nutritional component that could improve stress levels. It is not completely clear that bleaching is caused by a ROS-driven mechanism, as while some studies support an oxidative-stress-linked models (e.g., Downs et al., 2002), other alternative mechanistic pathways have been proposed (Dungan et al., 2022). Nonetheless, antioxidant-rich feeds remain of applied interest, and Spirulina (*Limnospira*/*Arthrospira*) contains phycobiliproteins and carotenoids with documented antioxidant activity (Spínola et al., 2024); experimental work suggests that preconditioning and nutritional context can shape post-stress outcomes (Majerová and Drury, 2022).

Here we evaluate whether feeding coral recruits heterotrophically with *Isochrysis* sp., or Spirulina, or a mixed diet influences recruit yield and growth during early post-settlement development. We also examine whether any dietary effects carry forward to a subsequent cumulative heatwave emulation. We focus on two Indo-Pacific *Acropora* species (*A. tenuis* and *A. digitifera*) commonly used in restoration and experimental studies, and test for species-specific nutritional responses relevant to the design of scalable ex situ propagation protocols.

## Materials and Methods

### Experimental design

Two reef-building coral species were used in this study, *Acropora tenuis* and *Acropora digitifera*, both of which are widespread Indo-Pacific corals commonly used in experimental studies of coral recruitment and restoration. Adult colonies of *Acropora tenuis* and *Acropora digitifera* were spawned at the Palau International Coral Reef Center (PICRC), Palau, at different time points. *A. tenuis* was spawned on March 18th, 2024, while *A. digitifera* was on April 18th, 2024. Following spawning, larvae were settled on conditioned frag plugs, and spats were kept in common tanks.

On May 26th, a total of 540 settlement plugs having coral recruits (~ 1 – 7 individuals on each plug), consisting of 360 plugs with *A. tenuis* recruits and 180 plugs with *A. digitifera* recruits, were exposed to four feeding treatments until August 4th (71 days) to assess the effect of heterotrophic feeding on yield and growth. The feeding treatments were composed of 1) microalgae Isochrysis galbana; 2) cyanobacterium Spirulina; 3) a mixture of Isochrysis and Spirulina, and 4) no food – unfed control. Each treatment was replicated three times, resulting in a total of 12 experimental tanks, where frag plugs were distributed evenly. Each tank contained 45 frag plugs, composed of a mixture of both species.

Each tank consisted of a 3 L flow-through aquarium supplied continuously with filtered seawater (20 µm), ensuring regular water turnover and avoiding heterotrophic feeding on wild phytoplankton or zooplankton. Additionally, a circulation pump was added to each tank to support water motion. Three sets of lights were set to ensure autotrophic feeding of corals and maintain basal metabolic needs. To control benthic and filamentous algal growth, each tank was stocked with 30 Trochus snails of different sizes, a density sufficient to maintain low background algal cover without disturbing coral recruits. Tanks were also scrubbed and cleaned once a week to remove accumulated biofilm and debris produced by snails.

### Feeding Procedures

Coral recruits were fed every other day for the duration of the experiment. During each feeding event, water flow to the tanks was kept on for the first 15 minutes to favor dilution, then flow was shut off for 2h to allow coral recruits to feed on the supplied food. Following the feeding period, water flow was restored to maintain water quality.

For the Spirulina treatment, a stock solution was prepared by dissolving 1 g of spirulina powder in 30 mL of seawater. This solution was homogenized, and each Spirulina treatment tank received 5 mL of the stock solution per feeding event. Tanks assigned to the mixed Isochrysis + Spirulina treatment received 2.5 mL of the same spirulina stock solution, along with Isochrysis culture at exactly half the volume administered to the Isochrysis-only treatment tanks on the same day.

For the Isochrysis treatment, algal cell concentrations were first quantified using a hemocytometer to generate calibration standards, which were then used to adapt the Secchi disk method for rapid and reliable cell density estimation. At each feeding day 1L of algae was visually assessed using the Secchi disk, and the depth at which the center of the disk became opaque was used to retrieve the cell's concentration. Based on this assessment, a consistent volume of Isochrysis culture was selected for each feeding session to maintain comparable algal availability across feeding events. To ensure that algae were supplied at consistent concentrations across tanks and feeding sessions, water samples were collected from each tank immediately after water flow was shut off during

feeding events and analyzed with a microscope (40X magnification). From each sample, one drop of water was poured on a hemocytometer, and the number of algal cells present (Isochrysis or Spirulina, depending on treatment) was counted. These counts were recorded to verify consistency in algal delivery among replicate tanks and across feeding sessions. Control tanks did not receive any supplemental algae and were maintained under identical conditions.

The inclusion of Spirulina, which contains high concentrations of antioxidant compounds such as phycocyanin and carotenoids, was intended to test 1) antioxidant-rich diets on survival and growth of juvenile corals; 2) any latent effect of antioxidant-rich diets on oxidative stress mitigation during thermal stress.

### Cumulative heatwave emulation experiment

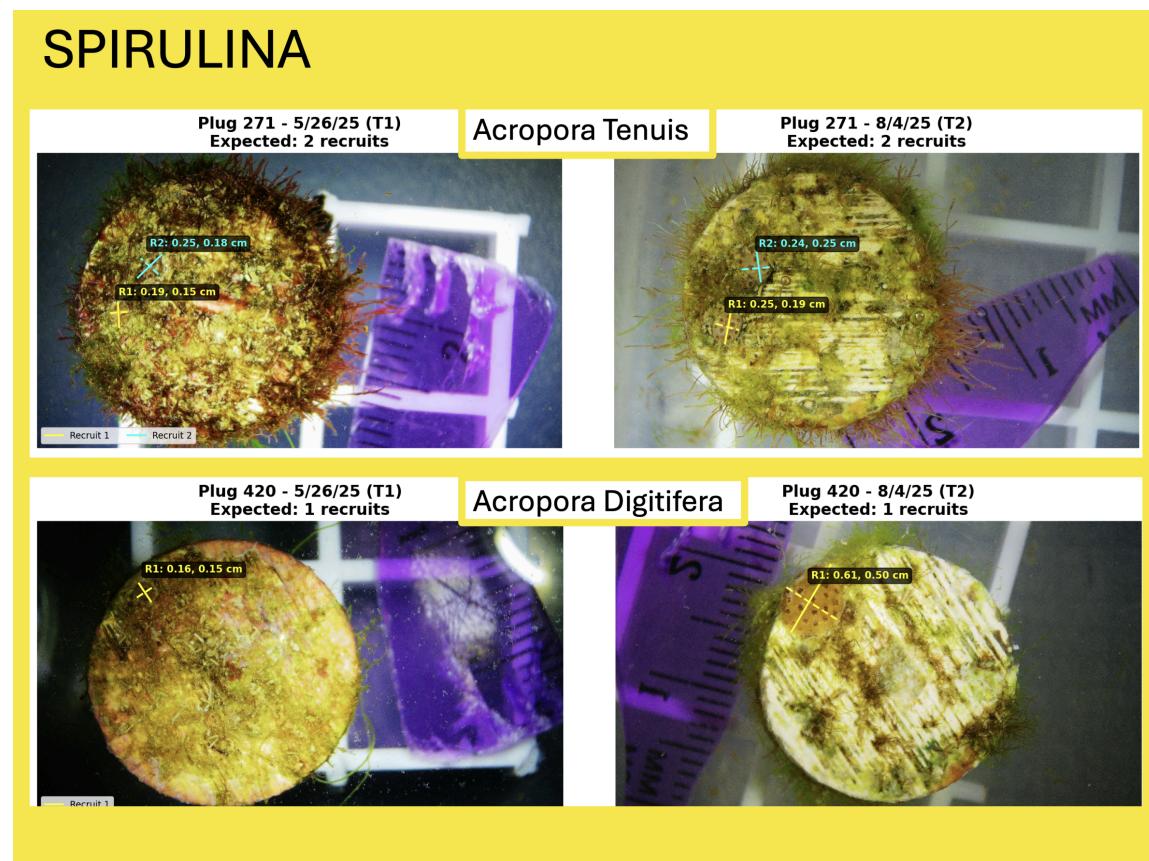
On August 18th, a total of 337 frag plugs from different feeding treatments, each with a single recruit per plug, were relocated evenly across four tanks and exposed to a cumulative heatwave emulation experiment for two weeks. Water inflow - directly connected to the aquarium's main pipeline - supplied all tanks to ensure good water quality, while temperature was regulated through independent heaters and controllers. This system allowed precise temperature control throughout the two-week marine heatwave emulation experiment following Lachs et al. (2023). Hobo pendant data loggers with a recording time set at a 10-minute interval were placed in each tank and allowed to calculate the accumulated heat over time. Degree Heating Weeks (DHW) is a thermal stress metric developed by NOAA to quantify accumulated heat exposure leading to coral bleaching ([https://coralreefwatch.noaa.gov/product/5km/index\\_5km\\_dhw.php](https://coralreefwatch.noaa.gov/product/5km/index_5km_dhw.php)). NOAA Coral Reef Watch (CRW) uses daily SST converted to temperature anomalies by subtracting the local maximum of monthly means climatology (MMM) and then transformed to be positive-only HotSpots. For our experiment, we adapted the DHW method by deriving the MMM from long-term in situ temperature measurements at the source reef of parental colonies following Humanes et al.(2024). The thermic ramp began at 32.5 °C and increased by 0.5 °C every two days, reaching 34.5 °C after nine days of the experiment.

### Data Collection

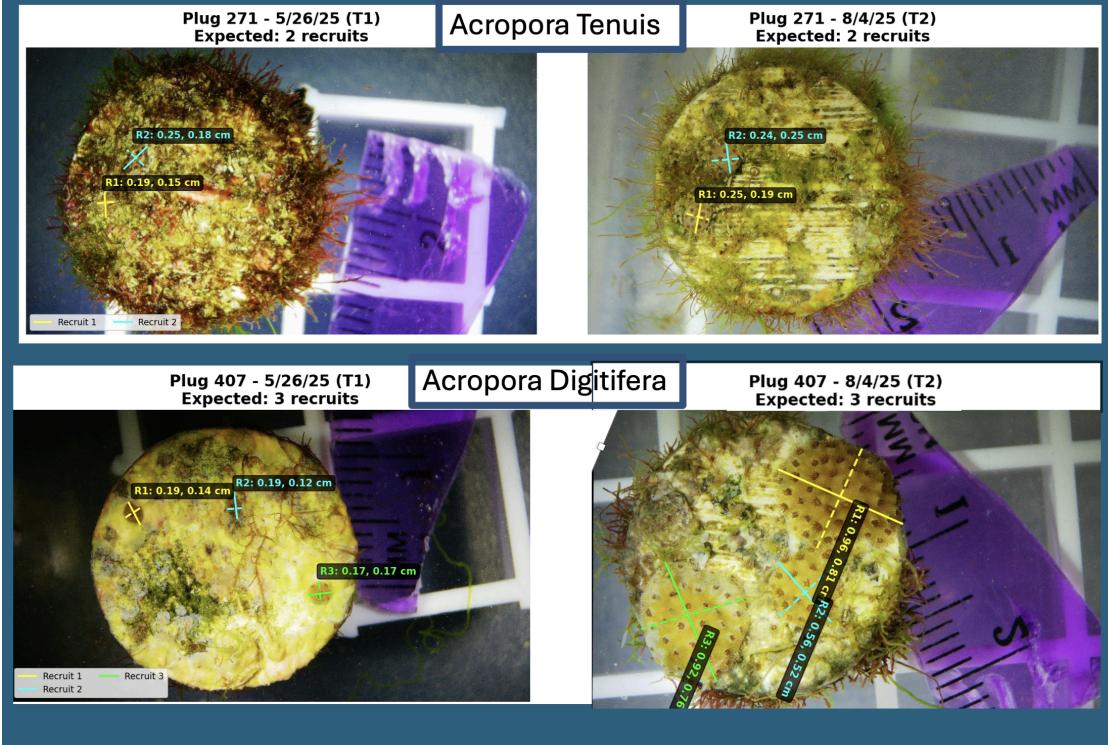
Recruits on frag plugs were surveyed once per month throughout the three-month experimental period. During each survey, the number of coral recruits present on each plug was counted and recorded. Final recruit counts were conducted in August at the conclusion of the experiment. To assess the effect of heterotrophic feeding on recruit survival, we analyzed the two species separately and modeled the yield (i.e., the ratio of surviving recruits at the end of the experiment to the initial number of recruits on each plug) against feeding treatments and added tanks as a random factor using a linear mixed effect model (i.e., function lmer in Rstudio).

To assess coral growth over time, photographs were taken of 10 settlement plugs per tank for each coral species at each time point (examples with measurements overlaid shown in Figure 1). Photos from the first time point (e.g., beginning of the experiment in May) and last time point (e.g. August) were analyzed with a custom interactive visualization and measurement software tool (PolypPal, Croft, 2024) written in Python. The tool loads images for each plug at both timepoints simultaneously, allows the user to rotate and zoom both images together, keeps track of recruit location and records measurements to a spreadsheet directly. Measurements were validated by direct comparison with ImageJ (e.g., Schneider et al., 2012) for a sample of ten plugs. The maximum and perpendicular maximum diameter of each juvenile were recorded at each time point. These two metrics were used to calculate the geometric mean diameter (GMD, as in Guest et al., 2014). Then, growth was calculated from the difference in the geometric mean diameter of each individual from the end and the beginning of the experiment, allowing comparisons of growth rates among treatments.

Bleaching and mortality response during the cumulative heatwave emulation experiment were assessed visually through daily surveys. During the daily surveys, each individual was scored as Healthy, Pale, Bleached, or Dead, and proportions for each category were noted.



# ISOCHRYYSIS



**Figure 1.** Representative settlement plug photographs illustrating the growth measurement approach. (Top image) Example Spirulina-fed plugs and recruits photographed at the start (T1; 26 May 2025) and end (T2; 4 Aug 2025) of the feeding phase, with two orthogonal diameter measurements per recruit overlaid. (Bottom image) Corresponding examples from the Isochrysis-only treatment. Images show one plug for each species per treatment as an illustration of measurement and scale.

## Statistical analysis

Growth was analyzed separately for each species using linear mixed-effects models with feeding treatment as a fixed effect and tank as a random intercept. Recruit size was quantified using the GMD from orthogonal diameter measurements on standardized images as described above; absolute growth was defined as final minus initial GMD. Model-based means and standard errors are reported for each treatment. Bleaching and mortality responses during the heatwave emulation were analyzed with time-to-event models, including treatment-specific effects on bleaching onset and progression, as described in the accompanying results text.

Three random effects structures were compared using AICc: tank-only, plug-only, and nested (plug within tank). For *A. tenuis*, Plug\_only and Nested models had equivalent support ( $\Delta\text{AICc} = 0.6$ ), with the simpler model preferred; tank-level variation was

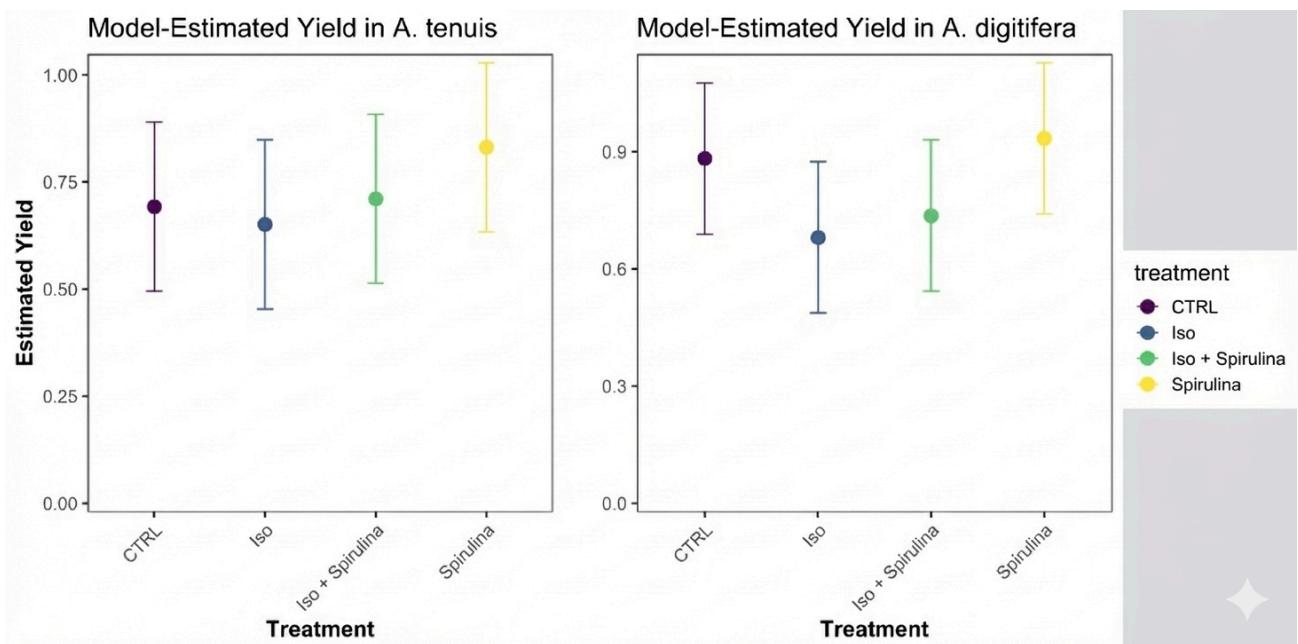
negligible after accounting for plug effects. For *A. digitifera*, the Nested model was strongly favored ( $\Delta\text{AICc} = 14.2$ ), indicating both clustering sources mattered. Model-based estimated marginal means from the selected models were used for all comparisons, with Tukey's HSD adjustment for multiple testing.

## Results

### Survival and growth

To confirm that treatment groups began the feeding phase with comparable recruit sizes, we tested for differences in initial geometric mean diameter (T1) among treatments using mixed-effects models. Initial sizes did not differ significantly among treatments for either species (*A. tenuis*: likelihood-ratio test  $p = 0.267$ ; *A. digitifera*:  $p = 0.346$ ; Fig. S1).

Recruit yield (the proportion of expected recruits still present at the end of the feeding phase) varied among diets, with Spirulina tending to produce the highest estimated yield in both species, while the Isochrysis-only diet tended to be lower (Fig. 1).

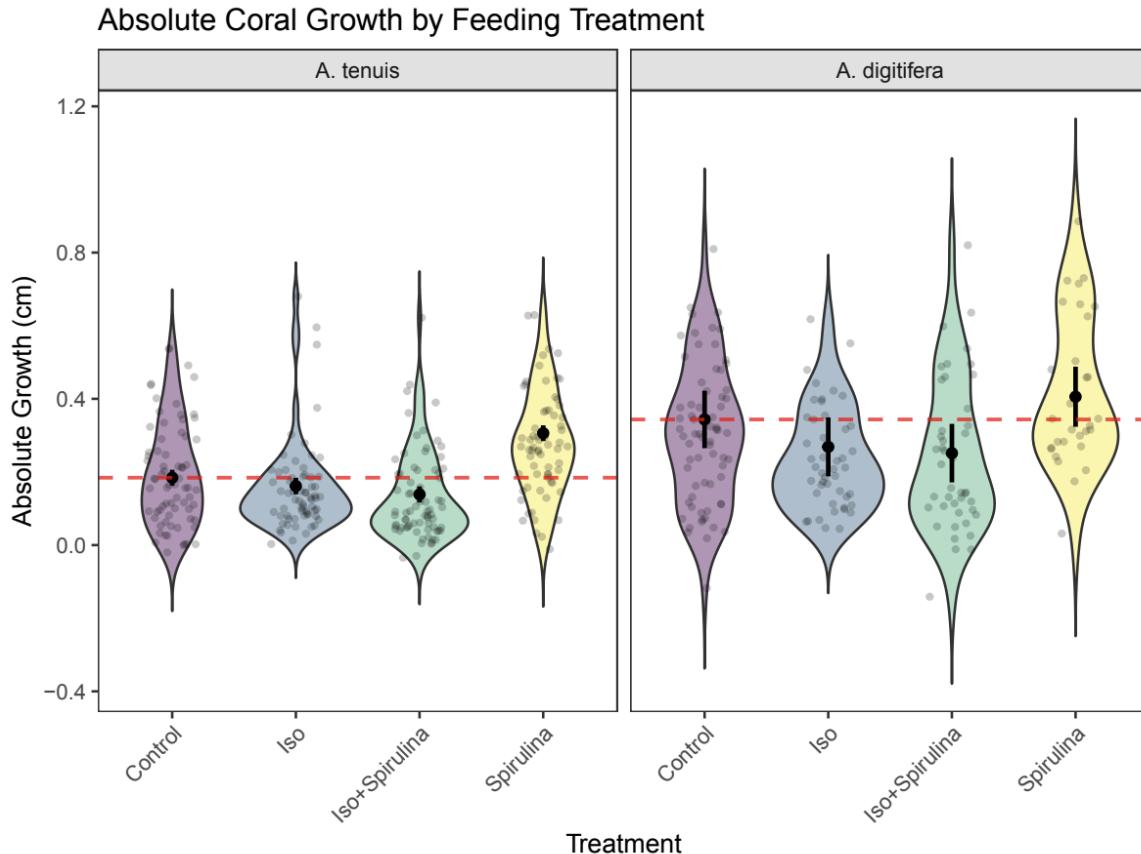


**Figure 2.** Model-estimated recruit yield for (left) *Acropora tenuis* and (right) *Acropora digitifera* across feeding treatments. Points show model-estimated means; whiskers indicate 95% confidence intervals.

**Table 1.** Model-based mean absolute growth (cm) during the feeding phase, estimated separately for each species. Values are estimated marginal means  $\pm$  SE with 95% confidence intervals (CI); N is the number of recruits included in the growth models.

Species	Treatment	N	Init (cm)	SE	Final (cm)	SE	Abs (cm)	SE
<i>A. tenuis</i>	Control	72	0.1507 58	0.00 67	0.3420 03	0.02 01	0.1844 0.1616	0.02 0.02 16 26
<i>A. tenuis</i>	Isochrysis	65	0.1528	0.00	0.3069	0.02	0.1616	0.02 26
<i>A. tenuis</i>	Iso+Spirulina	74	0.1381 62	0.00 38	0.2705 60	0.01	0.1386	0.02 21
<i>A. tenuis</i>	Spirulina	66	0.1475	0.00	0.4249	0.02 00	0.3060	0.02 15
<i>A. digitifera</i>	Control	69	0.1766 64	0.00 63	0.4809 48	0.02	0.3437	0.07 86
<i>A. digitifera</i>	Isochrysis	46	0.1827	0.00	0.4199 21	0.02	0.2687	0.08 06
<i>A. digitifera</i>	Iso+Spirulina	43	0.1652 86	0.00 20	0.4052 20	0.03	0.2515	0.08 00
<i>A. digitifera</i>	Spirulina	30	0.1890 71	0.00 71	0.6041	0.03 86	0.4058	0.08 22

Absolute growth differed strongly among diets for *A. tenuis* (N = 277 recruits across 91 plugs). A mixed-effects model with plug as a random effect detected a significant overall treatment effect (Type III ANOVA:  $F = 12.47$ ,  $p = 9.66 \times 10^{-7}$ ). Spirulina-fed recruits showed the greatest growth (estimated mean  $0.306 \pm 0.022$  cm), exceeding unfed controls ( $0.184 \pm 0.022$  cm; Tukey-adjusted  $p = 0.0007$ ) as well as Isochrysis-only ( $0.162 \pm 0.023$  cm;  $p < 0.0001$ ) and the mixed Isochrysis + Spirulina diet ( $0.139 \pm 0.022$  cm;  $p < 0.0001$ ; Table 1; Fig. 3). In contrast, *A. digitifera* growth (N = 188 recruits across 86 plugs) did not differ significantly among feeding treatments ( $p = 0.368$ ), despite numerically higher mean growth under Spirulina ( $0.406 \pm 0.082$  cm) relative to controls ( $0.344 \pm 0.079$  cm; Table 1; Fig. 3) (representative images in Fig. 1).



**Figure 3.** Absolute recruit growth (change in geometric mean diameter, cm) during the feeding phase for each treatment and species. Violin plots show the distribution of recruit-level growth values; points show individual recruits. Black points with error bars indicate model-based means  $\pm$  SE.

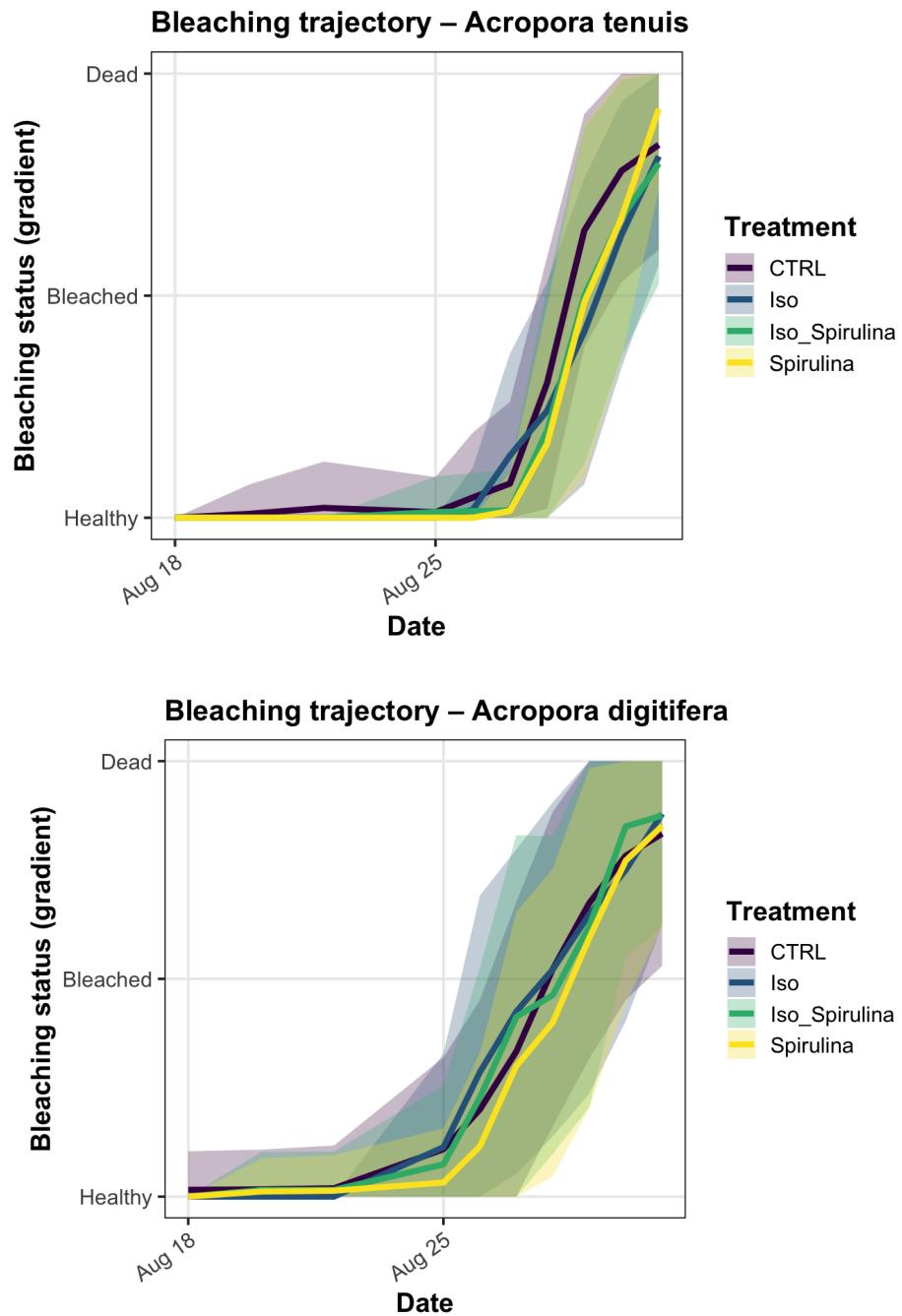
### Cumulative heatwave emulation experiment

Following the feeding phase, a subset of recruits was subjected to a controlled cumulative heatwave emulation designed to mimic a marine heatwave profile and to enable time-resolved quantification of bleaching and mortality.

### Bleaching response

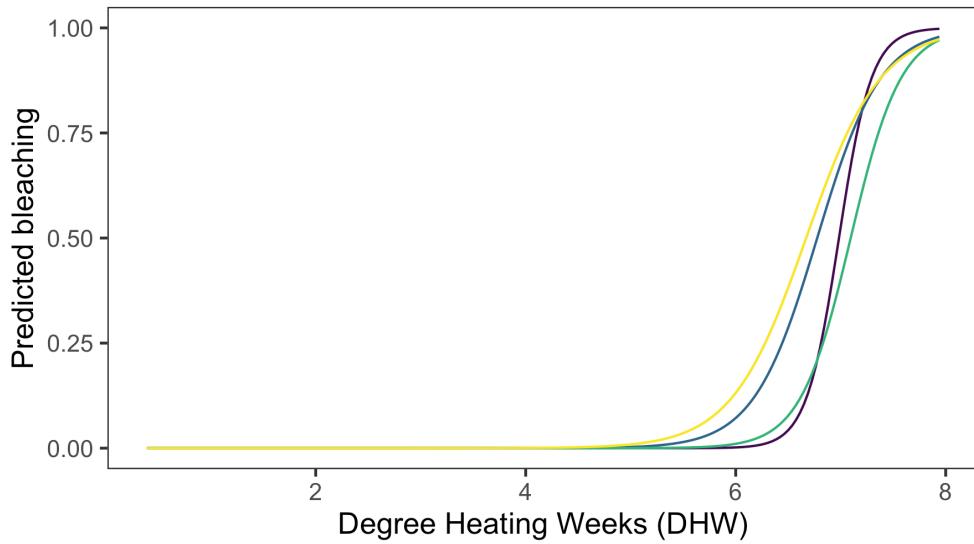
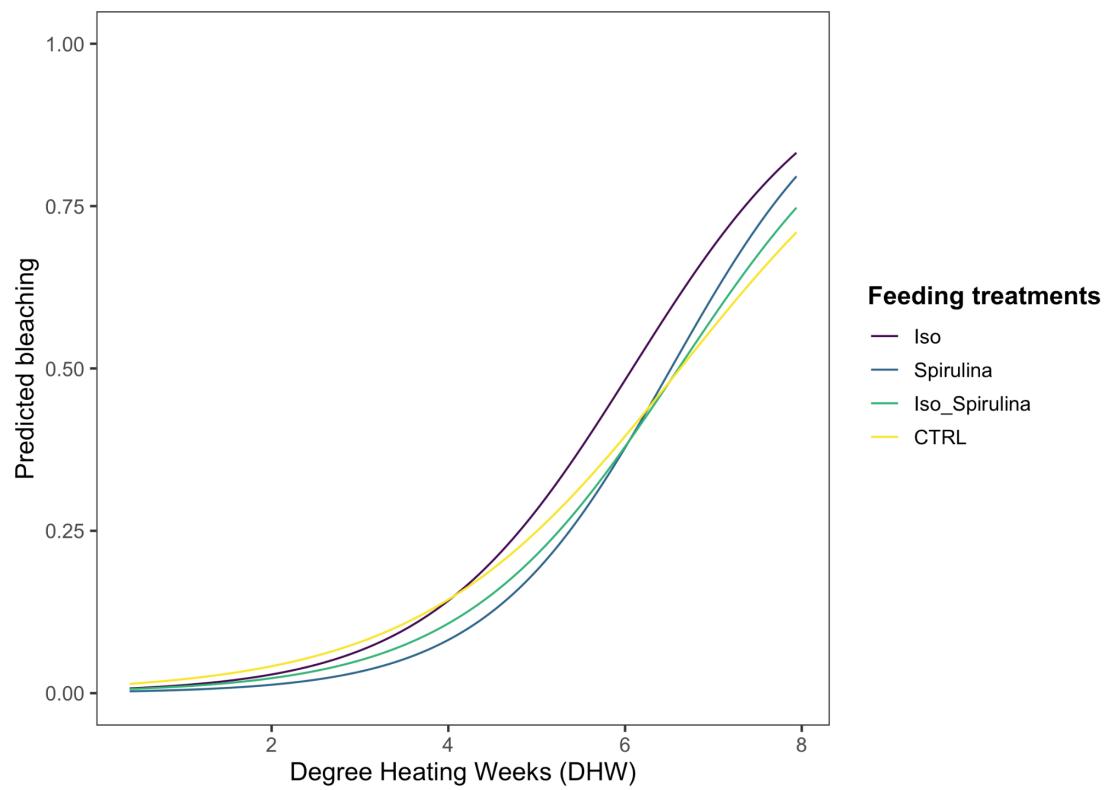
In *Acropora tenuis*, bleaching probability among surviving tissue increased sharply with cumulative thermal stress (Degree Heating Weeks, DHW;  $z = 77.4$ ,  $p < 0.001$ ). Feeding treatments significantly reduced baseline bleaching probability at low DHW, indicating delayed bleaching onset, with the strongest effect observed under Spirulina feeding. However, feeding significantly altered bleaching sensitivity to DHW, as evidenced by positive DHW  $\times$  treatment interactions. Isochrysis ( $z = 8.06$ ,  $p < 0.001$ ), Isochrysis–Spirulina ( $z = 16.47$ ,  $p < 0.001$ ), and Spirulina ( $z = 25.19$ ,  $p < 0.001$ ) all exhibited steeper bleaching–DHW relationships than unfed controls. These results indicate that heterotrophic feeding delays the onset of bleaching but accelerates bleaching progression under sustained thermal stress. (Fig. 4A; Fig. 5A).

In *Acropora digitifera*, bleaching probability among surviving tissue increased strongly with cumulative thermal stress (Degree Heating Weeks, DHW;  $z = 53.7$ ,  $p < 0.001$ ). Feeding treatments had relatively modest effects on baseline bleaching probability; Isochrysis feeding slightly increased bleaching at low DHW ( $z = 2.04$ ,  $p = 0.041$ ), while the other treatments did not differ significantly from controls. However, feeding significantly modified bleaching sensitivity to thermal stress. Both Isochrysis ( $z = -4.31$ ,  $p < 0.001$ ) and Isochrysis–Spirulina ( $z = -3.70$ ,  $p < 0.001$ ) treatments reduced the rate at which bleaching increased with DHW, indicating partial buffering of bleaching progression under heat stress. In contrast, Spirulina feeding did not significantly affect bleaching sensitivity. (Fig. 4B; Fig. 5B).



**Figure 4.** Observed bleaching trajectories during the cumulative heatwave emulation experiment. (Bottom) *Acropora digitifera* and (Top) *Acropora tenuis*. Lines show the mean bleaching status (Healthy → Bleached → Dead) through time for each feeding history; shaded regions indicate uncertainty around the mean.

### Predicted Bleaching in *A. digitifera*

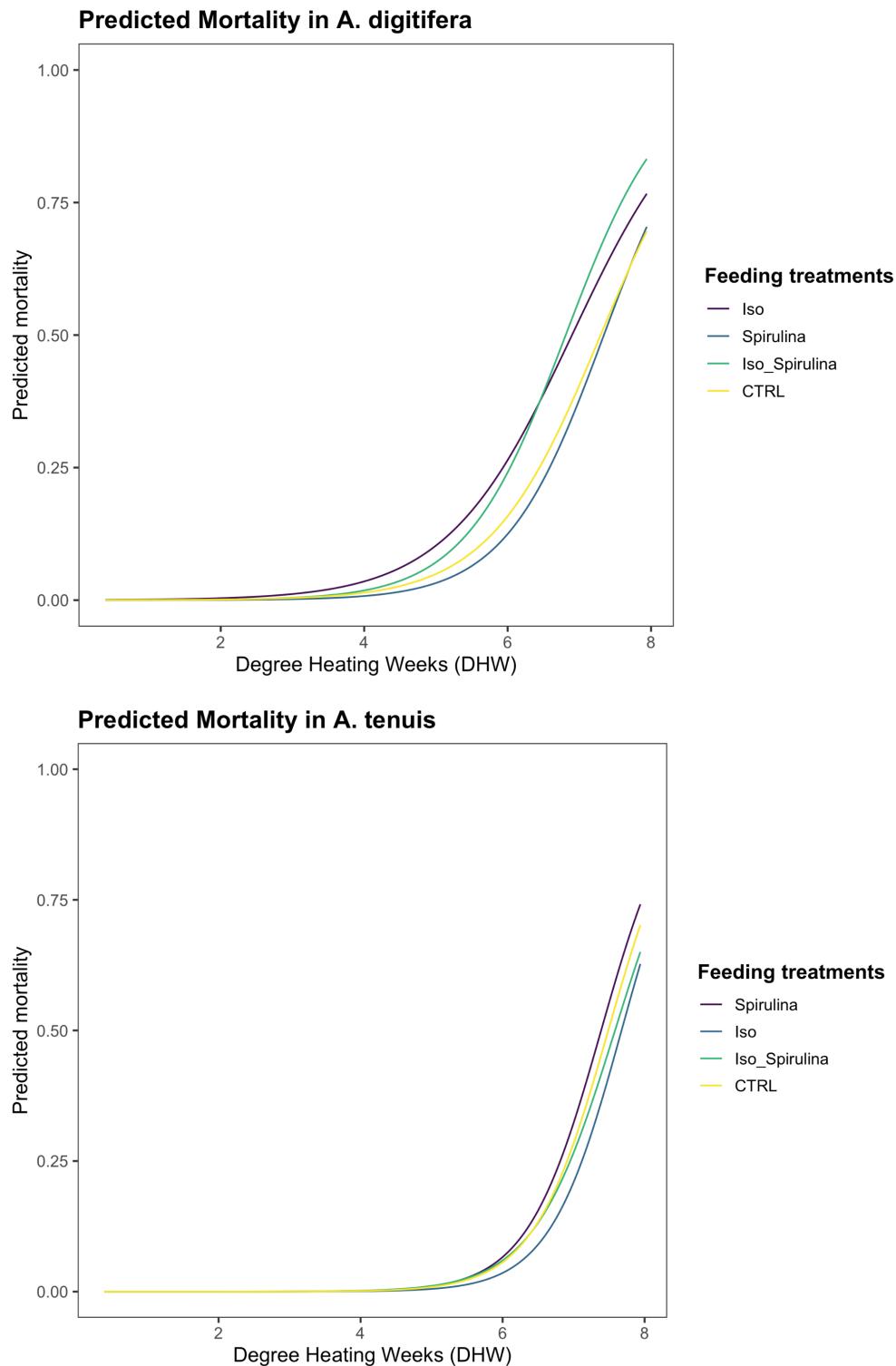


**Feeding treatments** — Spirulina — Iso — Iso\_Spirulina — CTRL

**Figure 5.** Model-predicted probability of bleaching as a function of cumulative thermal stress (Degree Heating Weeks, DHW) for (Top) *Acropora digitifera* and (Bottom) *Acropora tenuis*, stratified by feeding history. Curves are predictions from the fitted statistical models used to analyze bleaching response.

### Mortality response

In *Acropora tenuis*, mortality increased strongly with cumulative thermal stress (Degree Heating Weeks, DHW;  $z = 66.7$ ,  $p < 0.001$ ). Feeding treatment significantly modified both baseline mortality risk and the rate at which mortality accumulated with increasing DHW. Isochrysis feeding significantly reduced baseline mortality relative to unfed controls ( $z = -7.28$ ,  $p < 0.001$ ), but was associated with a steeper increase in mortality under thermal stress, as indicated by a positive DHW  $\times$  treatment interaction ( $z = 10.12$ ,  $p < 0.001$ ). In contrast, Spirulina feeding increased baseline mortality ( $z = 3.45$ ,  $p < 0.001$ ) but significantly reduced the slope of the mortality–DHW relationship ( $z = -6.24$ ,  $p < 0.001$ ), indicating improved tolerance to accumulating heat stress. The combined Isochrysis–Spirulina treatment did not significantly alter mortality patterns relative to controls. (Fig. 6A).



**Figure 6.** Model-predicted probability of mortality as a function of cumulative thermal stress (Degree Heating Weeks, DHW) for (Top) *Acropora digitifera* and (Bottom) *Acropora tenuis*, stratified by feeding history. Curves are predictions from the fitted statistical models used to analyze mortality response.

In *Acropora digitifera*, mortality also increased sharply with cumulative thermal stress (DHW;  $z = 49.8$ ,  $p < 0.001$ ). Feeding treatments had strong and contrasting effects on both baseline mortality and thermal sensitivity. Isochrysis and Spirulina feeding significantly increased baseline mortality relative to controls ( $p < 0.001$ ), yet both treatments significantly reduced the rate at which mortality increased with DHW (Isochrysis:  $z = -13.19$ ; Spirulina:  $z = -12.27$ ;  $p < 0.001$ ), indicating enhanced tolerance to prolonged thermal stress. In contrast, the combined Isochrysis–Spirulina treatment significantly reduced baseline mortality ( $z = -3.17$ ,  $p = 0.002$ ) but was associated with a steeper increase in mortality with DHW ( $z = 9.44$ ,  $p < 0.001$ ). (Fig. 6B).

Together, these species-specific models demonstrate that heterotrophic feeding alters both baseline survival and thermal sensitivity in corals, with the direction and magnitude of these effects strongly dependent on species identity and diet composition.

## Discussion

The feeding experiment shows that modest changes to the particulate food environment can measurably alter early post-settlement performance, but that effects are strongly species-dependent. The clearest pattern was in *A. tenuis*, where Spirulina supplementation increased absolute growth by  $\sim 0.12$  cm relative to unfed controls over 70 d, whereas Isochrysis-only and mixed diets did not outperform controls. In contrast, *A. digitifera* showed no detectable growth response to diet at the same feeding frequency, and stress responses during the cumulative-heatwave emulation differed among diets in ways that were not consistent across species. Together, these outcomes emphasize that “one-size-fits-all” feeding prescriptions are unlikely to apply across taxa, and that nutritional interventions should be tailored to the biology of the target species and specific aim (growth, survival, or stress tolerance).

Spirulina (*Arthrospira spp.*) is widely used as an aquaculture feed because it is protein-rich and contains bioactive pigments (including phycocyanin) and carotenoids that can function as antioxidants (Podgórska-Kryszczuk et al., 2024). Although our experiment was motivated in part by antioxidant hypotheses, the growth response we observed in *A. tenuis* could plausibly arise from multiple, non-mutually exclusive nutritional mechanisms. First, Spirulina may have provided limiting macronutrients (e.g., amino acids and lipids) that recruits could assimilate efficiently during a period when energy demands are high for calcification, tissue growth, and symbiosis establishment. Recent work on coral early life stages demonstrates that specific lipid classes—including sterols—can be critical for larval survival, swimming capacity, settlement performance, and thermal tolerance, highlighting that quality of nutrition and not only quantity, matters (Matthews et al., 2025). Second, Spirulina particles may differ in size, density, or buoyancy relative to Isochrysis in ways that increase encounter rates and capture

efficiency for small recruits under our 30-min no-flow feeding protocol, consistent with the broader literature emphasizing feeding mode and particle characteristics (Houlbrèque and Ferrier-Pagès, 2009). Third, Spirulina supplementation could have indirectly altered the microbial environment (e.g., via dissolved organics or associated microbial consortia), which in turn may influence nutrient acquisition or perhaps even early disease risk. This pathway is increasingly recognized as important but remains difficult to diagnose without targeted microbial and water-quality measurements.

Our study has several limitations that define priorities for future work. First, while we verified algal delivery via microscopic counts, we did not directly quantify nutrient composition (e.g., protein, lipid classes, sterols) or antioxidant capacity of the delivered diets, nor did we measure recruit energetic reserves (lipids, carbohydrates) during the growth phase. Second, the visual opacity (Secchi disk) approach for Isochrysis dosing, while practical, could introduce day-to-day variability, and future experiments could instead standardize microalgal rations using cell counts or carbon-equivalent dosing. Third, the mixed Isochrysis+Spirulina treatment underperformed in *A. tenuis*, which may reflect a dilution of an effective component, altered particle dynamics, or water-quality effects (e.g., microbial oxygen demand). This could be evaluated with measurements of dissolved oxygen, nutrients, and microbial abundance. Finally, linking diet to downstream thermal resilience will likely require a mechanistic explanation. This could be sought through measurements of symbiont density and photophysiology, host antioxidant enzyme activity, and tissue redox state. The measurements could distinguish whether diets act primarily through energy reserves, antioxidant provisioning, or microbial mediation.

From an applied perspective, the Spirulina growth response in *A. tenuis* suggests a low-complexity intervention that could increase production efficiency in ex situ coral nurseries. Even modest increases in early size can translate into higher handling tolerance, earlier eligibility for outplanting, and potentially improved post-outplant survival in high-disturbance environments. These findings complement recent work showing that targeted nutritional supplements can improve early survival and performance in larval and recruit culture, and that including larval feeding protocols can enhance settlement (Rodd et al., 2022). At the same time, our species-specific results reinforce that restoration pipelines should validate diet interventions on the focal species rather than assuming transferability from other taxa or life stages (Conlan et al., 2017; Cooper et al., 2025).

Spirulina was included as an antioxidant-rich supplement based on its pigment composition and antioxidant activity seen in other systems. Heat stress can disrupt cellular homeostasis and shift nutrient cycling before visible bleaching occurs (Rädecker et al., 2021), and early hypotheses proposed that ROS production and antioxidant limitation could be central drivers of symbiosis breakdown (Downs et al., 2002; Lesser,

2006). At the same time, recent experimental work cautions that oxidative-stress signatures do not always map cleanly onto bleaching outcomes, suggesting that ROS may be one of several pathways to symbiosis failure rather than a universal proximal cause (Dungan et al., 2022). Our results are consistent with this mixed picture: while Spirulina improved *A. tenuis* growth under benign conditions, it did not yield a simple, across-the-board reduction in bleaching or mortality during thermal stress. One interpretation is that simply adding antioxidants to the diet may not be enough unless it actually changes the oxidative stress state within the coral's tissues. Achieving that might require a different dose, a more bioavailable form of delivery, or pairing antioxidant. Alternatively, dietary antioxidants may matter most for resilience and recovery after stress rather than for delaying onset. Such an idea is supported by work showing that preconditioning or nutritional history can alter post-stress trajectories (Majerová and Drury, 2022).

We also asked whether early diet history carried forward into altered sensitivity during a cumulative-heatwave emulation. The stress experiment confirmed that bleaching and mortality risk rose steeply with cumulative thermal stress (DHW), consistent with the broader bleaching literature (Eakin et al., 2019). However, dietary effects on bleaching probability and mortality were not uniformly protective and depended on species identity and response variable, indicating that faster early growth does not necessarily translate into lower acute thermal sensitivity. This aligns with growing evidence that energetic trade-offs and stress responses can be complex: heterotrophic feeding can bolster energetic reserves and support recovery in some contexts, but outcomes depend on the interaction between diet composition, baseline physiological state, and the dominant mechanism of stress injury (Grottoli et al., 2006; Rädecker et al., 2021).

Why did *A. digitifera* not show a statistically detectable growth response under the same feeding schedule? Species can differ in polyp morphology, particle-capture capacity, reliance on heterotrophy, and the timing of symbiont acquisition. Any of these may shift the balance between autotrophic and heterotrophic inputs during early development (Houlbrèque and Ferrier-Pagès, 2009). In addition, our mixed-effects models indicate substantial among-tank and among-plug variance components for *A. digitifera* growth, which can reduce power to detect treatment effects even when mean differences are suggestive (Table 1). These considerations point to at least two practical next steps: (i) quantify feeding behavior directly (particle capture rates, gut content, or stable-isotope tracers) to evaluate whether *A. digitifera* recruits simply ingest less of the provided diets under the experimental hydrodynamics; and (ii) test dose-response and timing, as the “effective” feeding window and required food concentration may differ between species.

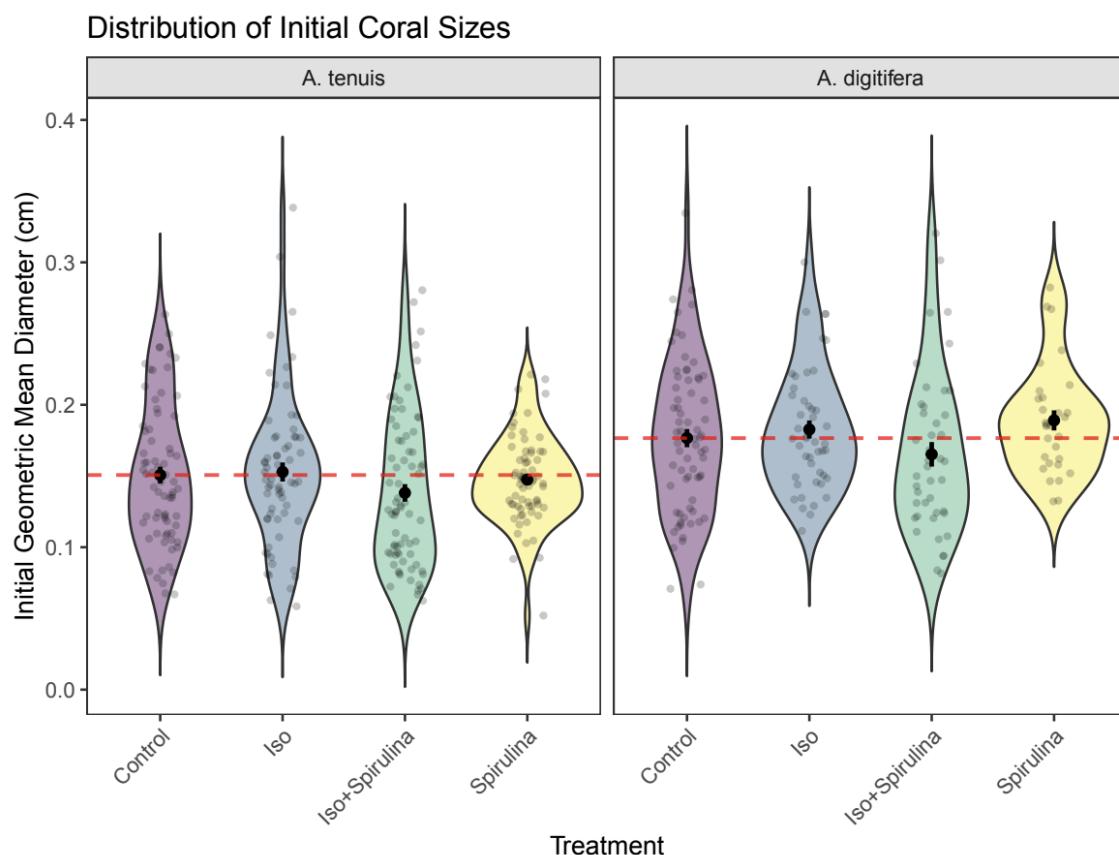
## Conclusions

Microalgal supplementation during early grow-out altered recruit growth and, in *A. tenuis*, influenced the dynamics of bleaching under cumulative heat stress. Spirulina-fed recruits exhibited the highest mean growth in both species, but feeding history did not detectably change final mortality during the heatwave emulation. Our findings suggest that practical, scalable dietary supplementation can improve early growth, but optimizing for growth alone may not translate into improved survival during heat stress. Resilience outcomes may depend on species, baseline condition, and the specific physiological pathways targeted by the supplement..

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## Supplementary material



**Figure S1.** Distribution of initial recruit sizes (geometric mean diameter at T1) by treatment for each species. Points represent individual recruits; black points with error bars indicate treatment means  $\pm$  SE; the dashed line indicates the overall mean.

## References

- Buerger, P., Alvarez-Rocha, P., Coppin, C. W., Pearce, S. L., Chakravarti, L. J., Oakeshott, J. G., Edwards, O. R., & van Oppen, M. J. H. (2020). Heat-evolved microalgal symbionts increase coral bleaching tolerance. *Science Advances*, 6(20), eaba2498. <https://doi.org/10.1126/sciadv.aba2498>
- Conlan, J. A., Humphrey, C. A., Severati, A., & Francis, D. S. (2017). Influence of different feeding regimes on the survival, growth, and biochemical composition of *Acropora* coral recruits. *PLOS ONE*, 12(11), e0188568. <https://doi.org/10.1371/journal.pone.0188568>
- Conlan, J. A., Jones, P. L., Browne, N. K., & Negri, A. P. (2019). Elucidating an optimal diet for captive *Acropora* corals. *Aquaculture*, 513, 734420. <https://doi.org/10.1016/j.aquaculture.2019.734420>
- Cooper, J. M., Kyprianou, H., & Leonhardt, J. (2025). Improving ex situ coral culture outcomes: Growth and survival responses of *Galaxea fascicularis* recruits to supplemental feeding. *Frontiers in Marine Science*. Advance online publication. <https://doi.org/10.3389/fmars.2025.1728004>
- Croft, N. (2024). PolypPal: Interactive visualization and measurement tool for coral recruit analysis [Computer software]. GitHub. <https://github.com/NicolasCroft/PolypPal>
- Downs, C. A., Fauth, J. E., Halas, J. C., Dustan, P., Bemiss, J., & Woodley, C. M. (2002). Oxidative stress and seasonal coral bleaching. *Free Radical Biology and Medicine*, 33(4), 533–543. [https://doi.org/10.1016/S0891-5849\(02\)00907-3](https://doi.org/10.1016/S0891-5849(02)00907-3)
- Dungan, A. M., Maire, J., Perez-Gonzalez, A., Blackall, L. L., & van Oppen, M. J. H. (2022). Lack of evidence for the oxidative stress theory of bleaching in the sea anemone *Exaiptasia diaphana* under elevated temperature. *Coral Reefs*, 41, 1161–1172. <https://doi.org/10.1007/s00338-022-02251-w>
- Eakin, C. M., Sweatman, H. P. A., & Brainard, R. E. (2019). The 2014–2017 global-scale coral bleaching event: insights and impacts. *Coral Reefs*, 38, 539–545. <https://doi.org/10.1007/s00338-019-01844-2>
- Grottoli, A. G., Rodrigues, L. J., & Palardy, J. E. (2006). Heterotrophic plasticity and resilience in bleached corals. *Nature*, 440, 1186–1189. <https://doi.org/10.1038/nature04565>
- Guest, J. R., Baria, M. V., Gomez, E. D., Heyward, A. J., & Edwards, A. J. (2014). Closing the circle: is it feasible to rehabilitate reefs with sexually propagated corals? *Coral Reefs*, 33, 45–55. <https://doi.org/10.1007/s00338-013-1114-1>

Houlbrèque, F., & Ferrier-Pagès, C. (2009). Heterotrophy in tropical scleractinian corals. *Biological Reviews*, 84(1), 1–17. <https://doi.org/10.1111/j.1469-185X.2008.00058.x>

Lachs, L., Humanes, A., Pygas, D. R., Bythell, J. C., Mumby, P. J., Ferrari, R., Figueira, W. F., Beauchamp, E., East, H. K., Edwards, A. J., Golbuu, Y., Martinez, H. M., Sommer, B., van der Steeg, E., & Guest, J. R. (2023). No apparent trade-offs associated with heat tolerance in a reef-building coral. *Communications Biology*, 6(1), 400. <https://doi.org/10.1038/s42003-023-04758-6>

Leal, M. C., Ferrier-Pagès, C., Calado, R., Thompson, M. E., Frischer, M. E., & Nejstgaard, J. C. (2014). Coral feeding on microalgae assessed with molecular trophic markers. *Molecular Ecology*, 23(15), 3870–3876. <https://doi.org/10.1111/mec.12486>

Hughes, T. P., Kerry, J. T., Álvarez-Noriega, M., et al. (2017). Global warming and recurrent mass bleaching of corals. *Nature*, 543, 373–377. <https://doi.org/10.1038/nature21707>

Humanes, A., Lachs, L., Buerger, P., et al. (2024). Selective breeding enhances coral heat tolerance to marine heatwaves, but tradeoffs remain. *Nature Communications*, 15, 1380. <https://doi.org/10.1038/s41467-024-45608-9>

Krueger, T., Hawkins, T. D., Becker, S., et al. (2015). Differential coral bleaching—Contrasting the activity and response of enzymatic antioxidants in symbiotic partners under thermal stress. *Comparative Biochemistry and Physiology Part A*, 190, 15–25. <https://doi.org/10.1016/j.cbpa.2015.08.012>

Lachs, L., Buerger, P., & van Oppen, M. J. H. (2023). Coral interventions can delay onset but accelerate progression of heat-induced bleaching. *Communications Biology*, 6, 1142. <https://doi.org/10.1038/s42003-023-05432-1>

Lesser, M. P. (2006). Oxidative stress in marine environments: biochemistry and physiological ecology. *Annual Review of Physiology*, 68, 253–278. <https://doi.org/10.1146/annurev.physiol.68.040104.110001>

Majerová, E., & Drury, C. (2022). Thermal preconditioning in a reef-building coral alleviates oxidative damage through a BI-1-mediated antioxidant response. *Frontiers in Marine Science*, 9, 971332. <https://doi.org/10.3389/fmars.2022.971332>

Matthews, J. L., van Oppen, M. J. H., Buerger, P., et al. (2025). Sterols are key to coral larvae survival, swimming capacity, and thermal tolerance. *Communications Biology*, 8, 1494. <https://doi.org/10.1038/s42003-025-08965-1>

Rädecker, N., Pogoreutz, C., Voolstra, C. R., Wiedenmann, J., & Wild, C. (2021). Heat stress destabilizes symbiotic nutrient cycling in corals. *Proceedings of the National Academy of Sciences*, 118(5), e2022653118. <https://doi.org/10.1073/pnas.2022653118>

Rodd, S. L., Whalan, S., & Harrison, P. L. (2022). Enhancing coral settlement through a novel larval feeding protocol. *Frontiers in Marine Science*, 9, 918232. <https://doi.org/10.3389/fmars.2022.918232>

Rodd, S. L., Whalan, S., & Harrison, P. L. (2025). Culturing coral larvae with food supplements enhances larval size and settlement. *Aquaculture*, 597, 742392. <https://doi.org/10.1016/j.aquaculture.2024.742392>

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671–675. <https://doi.org/10.1038/nmeth.2089>

Skirving, W., Heron, S. F., Liu, G., et al. (2020). CoralTemp and the Coral Reef Watch Coral Bleaching Heat Stress Product Suite version 3.1. *Remote Sensing*, 12(23), 3856. <https://doi.org/10.3390/rs12233856>

Suggett, D. J., & Smith, D. J. (2020). Coral bleaching patterns are the outcome of complex biological and environmental networks. *Global Change Biology*, 26, 68–79. <https://doi.org/10.1111/gcb.14871>

Spínola, M. P., Mendes, A. R., & Prates, J. A. M. (2024). Chemical composition, bioactivities, and applications of Spirulina (*Limnospira platensis*) in food, feed, and medicine. *Foods*, 13(22), 3656. <https://doi.org/10.3390/foods13223656>